

contiguous amino acid residues thereof, wherein said *hedgehog* polypeptide binds to a naturally occurring *patched* receptor, and wherein said amount of the *hedgehog* polypeptide is effective to promote one or more of growth, differentiation, and survival of said cells in culture.

Part 07
M

124. **(Twice Amended)** A method for promoting survival of mammalian neuronal cells responsive to *hedgehog* induction, comprising treating the cells in culture with an effective amount of a *hedgehog* polypeptide at least 80% identical to at least one sequence selected from SEQ ID NO: 8, SEQ ID NO: 11, SEQ ID NO: 12, SEQ ID NO: 13, or an N-terminal fragment of at least 50 contiguous amino acid residues thereof, wherein said *hedgehog* polypeptide binds to a naturally occurring *patched* receptor, and wherein said amount of the *hedgehog* polypeptide is effective to increase the survival of the neuronal cells in culture.

125. **(Twice Amended)** A method for promoting growth of mammalian neuronal stem cells in culture, comprising treating the cells with an amount of a *hedgehog* polypeptide at least 80% identical to at least one sequence selected from SEQ ID NO: 8, SEQ ID NO: 11, SEQ ID NO: 12, SEQ ID NO: 13, or an N-terminal fragment of at least 50 contiguous amino acid residues thereof, wherein said *hedgehog* polypeptide binds to a naturally occurring *patched* receptor, and wherein the amount of the *hedgehog* polypeptide is effective to increase the rate of growth of the neuronal stem cells in culture.

126. **(Reiterated)** The method of any one of claims 123, 124, or 125, wherein said *hedgehog* polypeptide is administered in combination with one or more other neurotrophic factors.

127. **(Reiterated)** The method of claim 126, wherein said other neurotrophic factor is selected from CNTF, BNTF, and NGF.

128. **(Reiterated)** The method of claim 123, wherein said neuronal cells are neural progenitor cells.

129. **(Reiterated)** The method of claim 123, wherein said neuronal cells differentiates into cells having a selected neural phenotype.

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130. (Amended) The method of claim 129, wherein said neuronal cells differentiate into cells of the central nervous system or the peripheral nervous system.

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Part D
133. (Twice Amended) The method of claim 123, wherein said *hedgehog* polypeptide comprises an amino acid sequence identical with an amino acid sequence designated in one of SEQ ID NO: 8, SEQ ID NO: 11, SEQ ID NO: 12, SEQ ID NO: 13, or an N-terminal fragment of at least 50 contiguous amino acid residues thereof.

134. (Amended) The method of any one of claims 123, 124, or 125, wherein said *hedgehog* polypeptide has an amino acid sequence which is encoded by a nucleic acid which hybridizes under stringent conditions, including a wash step of 0.2 x SSC at 65 °C, to a nucleic acid sequence selected from SEQ ID NO: 1, SEQ ID NO: 4, SEQ ID NO: 5, and SEQ ID NO: 6.

135. (Twice Amended) The method of claim 123, wherein said *hedgehog* polypeptide is encoded by a nucleic acid which is at least 98% identical with at least one of a nucleic acid sequence designated in SEQ ID NO: 1, SEQ ID NO: 4, SEQ ID NO: 5, SEQ ID NO: 6, or an N-terminal fragment of at least 150 contiguous nucleotides thereof.

Part D
136. (Twice Amended) The method of claim 123, wherein said *hedgehog* polypeptide is encoded by a nucleic acid which is at least 90% identical with at least one of a nucleic acid sequence designated in SEQ ID NO: 1, SEQ ID NO: 4, SEQ ID NO: 5, SEQ ID NO: 6, or an N-terminal fragment of at least 150 contiguous nucleotides thereof.

137. (Twice Amended) The method of claim 123, wherein said *hedgehog* polypeptide is encoded by a nucleic acid which is at least 95% identical with at least one of a nucleic acid sequence designated in SEQ ID NO: 1, SEQ ID NO: 4, SEQ ID NO: 5, SEQ ID NO: 6, or an N-terminal fragment of at least 150 contiguous nucleotides thereof.

138. (Twice Amended) The method of claim 123, wherein said polypeptide includes a *hedgehog* amino acid sequence at least 90 percent identical with a sequence selected from residues 104-189 of SEQ ID NO: 8, residues 102-187 of SEQ ID NO: 11, or residues 101-186 of SEQ ID NO: 12.

139. (Amended) The method of claim 123, wherein said polypeptide includes a *hedgehog* amino acid sequence at least 90 percent identical with a sequence selected from residues 27-189 of SEQ ID NO: 8, residues 25-187 of SEQ ID NO: 11, or residues 24-186 of SEQ ID NO: 12.

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cont

140. (Twice Amended) The method of claim 123, wherein said polypeptide includes a *hedgehog* amino acid sequence at least 90 percent identical with an amino acid sequence selected from residues 27-425 of SEQ ID NO: 8, residues 25-437 of SEQ ID NO: 11, residues 24-418 of SEQ ID NO: 12, or residues 24-475 of SEQ ID NO: 13.

141. (Reiterated) The method of claim 123, wherein said polypeptide includes an amino acid sequence encoded by a naturally occurring vertebrate *hedgehog* gene.

142. (Reiterated) The method of claim 141, wherein said *hedgehog* gene is a mammalian *hedgehog* gene.

143. (Reiterated) The method of claim 142, wherein said *hedgehog* gene is a human *hedgehog* gene.

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cont'd

144. (Twice Amended) The method of claim 123, wherein said polypeptide includes an amino acid sequence which is encoded by at least a portion of a *hedgehog* gene of vertebrate origin selected from nucleotides 64-567 of SEQ ID NO: 1, nucleotides 73-561 of SEQ ID NO: 4, and nucleotides 70-558 of SEQ ID NO: 5.

M5

146. (Amended) The method of claim 123, wherein said polypeptide includes at least 150 contiguous amino acid residues of the N-terminal half of a *hedgehog* protein.

147. (Reiterated) The method of claim 123, wherein said polypeptide binds to a naturally occurring *patched* receptor.

148. (Reiterated) The method of claim 147, wherein said *patched* receptor is a *patched* receptor of a vertebrate organism.

149. (Reiterated) The method of claim 123, wherein said neuronal cells are selected from motor neurons, cholinergic neurons, dopaminergic neurons, serotonergic neurons and peptidergic neurons.

151. (Amended) The method of claim 123, wherein said polypeptide includes at least 50 contiguous amino acid residues of the N-terminal half of a *hedgehog* protein.

152. (Amended) The method of claim 123, wherein said polypeptide includes at least 100 contiguous amino acid residues of the N-terminal half of a *hedgehog* protein.

153. (Twice Amended) A method for promoting one or more of growth, differentiation, and survival of neuronal cells in culture, comprising contacting said cells with an amount of a *hedgehog* polypeptide encodable by a nucleic acid which hybridizes under stringent conditions, including a wash step of 0.2 x SSC at 65 °C, to a nucleic acid sequence designated in one of SEQ ID NO: 1, SEQ ID NO: 4, SEQ ID NO: 5, SEQ ID NO: 6, or an N-terminal fragment thereof of at least 150 contiguous nucleotides, wherein said *hedgehog* polypeptide binds to a naturally occurring *patched* receptor, and wherein said amount of a *hedgehog* polypeptide is effective to promote one or more of growth, differentiation, and survival of said cells in culture.

154. (Twice Amended) A method for promoting survival of mammalian neuronal cells responsive to *hedgehog* induction, comprising treating the cells in culture with an amount of a *hedgehog* polypeptide effective to promote the survival of neuronal cells in culture, wherein said *hedgehog* polypeptide is encodable by a nucleic acid which hybridizes under stringent conditions, including a wash step of 0.2 x SSC at 65 °C, to a nucleic acid sequence designated in one of SEQ ID NO: 1, SEQ ID NO: 4, SEQ ID NO: 5, SEQ ID NO: 6, or an N-terminal fragment thereof of at least 150 contiguous nucleotides, wherein said *hedgehog* polypeptide binds to a naturally occurring *patched* receptor.

155. (Twice Amended) A method for promoting growth of mammalian neuronal stem cells in culture, comprising treating the cells with an amount of a *hedgehog* polypeptide encoded by a nucleic acid which hybridizes under stringent conditions, including a wash step of 0.2 x SSC at 65 °C, to a nucleic acid sequence designated in one of SEQ ID NO: 1, SEQ ID NO: 4, SEQ ID

NO: 5, SEQ ID NO: 6, or an N-terminal fragment thereof of at least 150 contiguous nucleotides, wherein said *hedgehog* polypeptide binds to a naturally occurring *patched* receptor, and wherein said amount of a *hedgehog* polypeptide is effective to increase the rate of growth of the neuronal stem cells in culture.

156. **(Twice Amended)** The method of claim 125, wherein said polypeptide sequence comprises a polypeptide encoded by a nucleic acid which is at least 90% identical to a nucleic acid sequence designated in one of SEQ ID NO: 1, SEQ ID NO: 4, SEQ ID NO: 5, SEQ ID NO: 6, or an N-terminal fragment of 150 contiguous nucleotides thereof.

157. **(Twice Amended)** The method of claim 125, wherein said polypeptide sequence comprises a polypeptide encoded by a nucleic acid which is at least 95% identical to a nucleic acid sequence designated in one of SEQ ID NO: 1, SEQ ID NO: 4, SEQ ID NO: 5, SEQ ID NO: 6, or an N-terminal fragment of 150 contiguous nucleotides thereof.

158. **(Twice Amended)** The method of claim 125, wherein said polypeptide sequence comprises a polypeptide encoded by a nucleic acid which is at least 98% identical to a nucleic acid sequence designated in one of SEQ ID NO: 1, SEQ ID NO: 4, SEQ ID NO: 5, SEQ ID NO: 6, or an N-terminal fragment of 150 contiguous nucleotides thereof.

159. **(Twice Amended)** The method of claim 124, wherein said polypeptide sequence comprises a polypeptide encoded by a nucleic acid which is at least 90% identical to a nucleic acid sequence designated in one of SEQ ID NO: 1, SEQ ID NO: 4, SEQ ID NO: 5, or SEQ ID NO: 6, or an N-terminal fragment of 150 contiguous nucleotides thereof.

160. **(Twice Amended)** The method of claim 124, wherein said polypeptide sequence comprises a polypeptide encoded by a nucleic acid which is at least 95% identical to a nucleic acid sequence designated in one of SEQ ID NO: 1, SEQ ID NO: 4, SEQ ID NO: 5, SEQ ID NO: 6, or an N-terminal fragment of 150 contiguous nucleotides thereof.

161. **(Twice Amended)** The method of claim 124, wherein said polypeptide sequence comprises a polypeptide encoded by a nucleic acid which is at least 98% identical to a nucleic

acid sequence designated in one of SEQ ID NO: 1, SEQ ID NO: 4, SEQ ID NO: 5, SEQ ID NO: 6, or an N-terminal fragment of 150 contiguous nucleotides thereof.

162. (Reiterated) The method of claim 123, wherein the N-terminal fragment is approximately 19 kD.

163. (Reiterated) The method of claim 124, wherein the N-terminal fragment is approximately 19 kD.

164. (Reiterated) The method of claim 125, wherein the N-terminal fragment is approximately 19 kD.

Please add the following new claims:

165. (New) The method of claim 123, wherein said neuronal cells differentiate into glial cells.

166. (New) The method of claim 129, wherein said neuronal cells differentiate into glial cells.

The claims presented above incorporate changes as indicated by the marked-up versions below.

123. (Twice Amended) A method for promoting one or more of growth, differentiation, and survival of neuronal cells in culture, comprising contacting said cells with an amount of a *hedgehog* polypeptide at least 80% identical to at least one sequence selected from SEQ ID NO: 8, ~~SEQ ID NO: 9, SEQ ID NO: 10, SEQ ID NO: 11, SEQ ID NO: 12, SEQ ID NO: 13, SEQ ID NO: 14~~, or an N-terminal fragment of at least 50 contiguous amino acid residues thereof, wherein said *hedgehog* polypeptide binds to a naturally occurring *patched* receptor, and wherein said amount of the *hedgehog* polypeptide is effective to promote one or more of growth, differentiation, and survival of said cells in culture.

124. (Twice Amended) A method for promoting survival of mammalian neuronal cells responsive to *hedgehog* induction, comprising treating the cells in culture with an effective amount of a *hedgehog* polypeptide at least 80% identical to at least one sequence selected from

SEQ ID NO: 8, ~~SEQ ID NO: 9, SEQ ID NO: 10,~~ SEQ ID NO: 11, SEQ ID NO: 12, SEQ ID NO: 13, ~~SEQ ID NO: 14,~~ or an N-terminal fragment of at least 50 contiguous amino acid residues thereof, wherein said *hedgehog* polypeptide binds to a naturally occurring *patched* receptor, and wherein said amount of the *hedgehog* polypeptide is effective to increase the survival of the neuronal cells in culture.

125. (Twice Amended) A method for promoting growth of mammalian neuronal stem cells in culture, comprising treating the cells with an amount of a *hedgehog* polypeptide at least 80% identical to at least one sequence selected from SEQ ID NO: 8, ~~SEQ ID NO: 9, SEQ ID NO: 10,~~ SEQ ID NO: 11, SEQ ID NO: 12, SEQ ID NO: 13, ~~SEQ ID NO: 14,~~ or an N-terminal fragment of at least 50 contiguous amino acid residues thereof, wherein said *hedgehog* polypeptide binds to a naturally occurring *patched* receptor, and wherein the amount of the *hedgehog* polypeptide is effective to increase the rate of growth of the neuronal stem cells in culture.

130. (Amended) The method of claim 129 +23, wherein said neuronal cells differentiate into cells of are in the central nervous system or the peripheral nervous system.

133. (Twice Amended) The method of claim 123, wherein said *hedgehog* polypeptide comprises an amino acid sequence identical with an amino acid sequence designated in one of SEQ ID NO: 8, ~~SEQ ID NO: 9, SEQ ID NO: 10,~~ SEQ ID NO: 11, SEQ ID NO: 12, SEQ ID NO: 13, ~~SEQ ID NO: 14,~~ or a an N-terminal fragment of at least 50 contiguous amino acid residues thereof.

134. (Amended) The method of any one of claims 123, 124, or 125, wherein said *hedgehog* polypeptide has an amino acid sequence which is encoded by a nucleic acid which hybridizes under highly stringent conditions, including a wash step of 0.2 x SSC at 65 °C, to a nucleic acid sequence selected from SEQ ID NO: 1, ~~SEQ ID NO: 2, SEQ ID NO: 3,~~ SEQ ID NO: 4, SEQ ID NO: 5, and SEQ ID NO: 6 and ~~SEQ ID NO: 7.~~

135. (Twice Amended) The method of claim 123, wherein said *hedgehog* polypeptide is encoded by a nucleic acid which is at least 98% identical with at least one of a nucleic acid sequence designated in SEQ ID NO: 1, ~~SEQ ID NO: 3,~~ SEQ ID NO: 4, SEQ ID NO: 5, SEQ ID

NO: 6, SEQ ID NO: 7, or a an N-terminal fragment of at least 150 contiguous nucleotides thereof.

136. **(Twice Amended)** The method of claim 123, wherein said *hedgehog* polypeptide is encoded by a nucleic acid which is at least 90% identical with at least one of a nucleic acid sequence designated in SEQ ID NO: 1, SEQ ID NO: 3, SEQ ID NO: 4, SEQ ID NO: 5, SEQ ID NO: 6, SEQ ID NO: 7, or a an N-terminal fragment of at least 150 contiguous nucleotides thereof.

137. **(Twice Amended)** The method of claim 123, wherein said *hedgehog* polypeptide is encoded by a nucleic acid which is at least 95% identical with at least one of a nucleic acid sequence designated in SEQ ID NO: 1, SEQ ID NO: 3, SEQ ID NO: 4, SEQ ID NO: 5, SEQ ID NO: 6, SEQ ID NO: 7, or a an N-terminal fragment of at least 150 contiguous nucleotides thereof.

138. **(Twice Amended)** The method of claim 123, wherein said polypeptide includes a *hedgehog* amino acid sequence at least 90 percent identical with a sequence selected from residues 104-189 of SEQ ID NO: 8, residues 102-187 of SEQ ID NO: 9, residues 31-116 of SEQ ID NO: 10, residues 102-187 of SEQ ID NO: 11, or residues 101-186 of SEQ ID NO: 12.

139. **(Amended)** The method of claim 123, wherein said polypeptide includes a *hedgehog* amino acid sequence at least 90 percent identical with a sequence selected from residues 27-189 of SEQ ID NO: 8, residues 22-187 of SEQ ID NO: 9, residues 1-116 of SEQ ID NO: 10, residues 25-187 of SEQ ID NO: 11, or residues 24-186 of SEQ ID NO: 12.

140. **(Twice Amended)** The method of claim 123, wherein said polypeptide includes a *hedgehog* amino acid sequence at least 90 percent identical with an amino acid sequence selected from residues 27-425 of SEQ ID NO: 8, residues 22-396 of SEQ ID NO: 9, residues 1-336 of SEQ ID NO: 10, residues 25-437 of SEQ ID NO: 11, residues 24-418 of SEQ ID NO: 12, or residues 24-475 of SEQ ID NO: 13, or residues 1-312 of SEQ ID NO: 14.

144. **(Twice Amended)** The method of claim 123, wherein said polypeptide includes an amino acid sequence which is encoded by at least a portion of a *hedgehog* gene of vertebrate origin selected from nucleotides 64-567 of SEQ ID NO: 1, ~~nucleotides 64-561 of SEQ ID NO: 2, nucleotides 1-348 of SEQ ID NO: 3~~, nucleotides 73-561 of SEQ ID NO: 4, and nucleotides 70-558 of SEQ ID NO: 5.

146. **(Amended)** The method of claim 123, wherein said polypeptide includes at least 150 contiguous amino acid residues of the N-terminal half of a *hedgehog* protein.

151. **(Amended)** The method of claim 123, wherein said polypeptide includes at least 50 contiguous amino acid residues of the N-terminal half of a *hedgehog* protein.

152. **(Amended)** The method of claim 123, wherein said polypeptide includes at least 100 contiguous amino acid residues of the N-terminal half of a *hedgehog* protein.

153. **(Twice Amended)** A method for promoting one or more of growth, differentiation, and survival of neuronal cells in culture, comprising contacting said cells with an amount of a *hedgehog* polypeptide encodable by a nucleic acid which hybridizes under stringent conditions, including a wash step of 0.2 x SSC at 65 °C, to a nucleic acid sequence designated in one of SEQ ID NO: 1, ~~SEQ ID NO: 2, SEQ ID NO: 3~~, SEQ ID NO: 4, SEQ ID NO: 5, SEQ ID NO: 6, ~~SEQ ID NO: 7~~, or a an N-terminal fragment thereof of at least 150 contiguous nucleotides, wherein said *hedgehog* polypeptide binds to a naturally occurring *patched* receptor, and wherein said amount of a *hedgehog* polypeptide is effective to promote one or more of growth, differentiation, and survival of said cells in culture.

154. **(Twice Amended)** A method for promoting survival of mammalian neuronal cells responsive to *hedgehog* induction, comprising treating the cells in culture with an amount of a *hedgehog* polypeptide effective to promote the survival of neuronal cells in culture, wherein said *hedgehog* polypeptide is encodable by a nucleic acid which hybridizes under stringent conditions, including a wash step of 0.2 x SSC at 65 °C, to a nucleic acid sequence designated in one of SEQ ID NO: 1, ~~SEQ ID NO: 2, SEQ ID NO: 3~~, SEQ ID NO: 4, SEQ ID NO: 5, SEQ ID

NO: 6, SEQ ID NO: 7, or a an N-terminal fragment thereof of at least 150 contiguous nucleotides, wherein said *hedgehog* polypeptide binds to a naturally occurring *patched* receptor.

155. **(Twice Amended)** A method for promoting growth of mammalian neuronal stem cells in culture, comprising treating the cells with an amount of a *hedgehog* polypeptide encoded by a nucleic acid which hybridizes under stringent conditions, including a wash step of 0.2 x SSC at 65 °C, to a nucleic acid sequence designated in one of SEQ ID NO: 1, ~~SEQ ID NO: 2, SEQ ID NO: 3, SEQ ID NO: 4, SEQ ID NO: 5, SEQ ID NO: 6, SEQ ID NO: 7, or a an N-terminal~~ fragment thereof of at least 150 contiguous nucleotides, wherein said *hedgehog* polypeptide binds to a naturally occurring *patched* receptor, and wherein said amount of a *hedgehog* polypeptide is effective to increase the rate of growth of the neuronal stem cells in culture.

156. **(Twice Amended)** The method of claim 125, wherein said polypeptide sequence comprises a polypeptide encoded by a nucleic acid which is at least 90% identical to ~~all or a portion of~~ a nucleic acid sequence designated in one of SEQ ID NO: 1, ~~SEQ ID NO: 2, SEQ ID NO: 3, SEQ ID NO: 4, SEQ ID NO: 5, SEQ ID NO: 6, or an N-terminal fragment of 150 contiguous nucleotides thereof SEQ ID NO: 7.~~

157. **(Twice Amended)** The method of claim 125, wherein said polypeptide sequence comprises a polypeptide encoded by a nucleic acid which is at least 95% identical to ~~all or a portion of~~ a nucleic acid sequence designated in one of SEQ ID NO: 1, ~~SEQ ID NO: 2, SEQ ID NO: 3, SEQ ID NO: 4, SEQ ID NO: 5, SEQ ID NO: 6, or an N-terminal fragment of 150 contiguous nucleotides thereof SEQ ID NO: 7.~~

158. **(Twice Amended)** The method of claim 125, wherein said polypeptide sequence comprises a polypeptide encoded by a nucleic acid which is at least 98% identical to ~~all or a portion of~~ a nucleic acid sequence designated in one of SEQ ID NO: 1, ~~SEQ ID NO: 2, SEQ ID NO: 3, SEQ ID NO: 4, SEQ ID NO: 5, SEQ ID NO: 6, or an N-terminal fragment of 150 contiguous nucleotides thereof SEQ ID NO: 7.~~

159. **(Twice Amended)** The method of claim 124, wherein said polypeptide sequence comprises a polypeptide encoded by a nucleic acid which is at least 90% identical to ~~all or a~~